جامعة بغداد

كلية العلوم

قسم التقنيات الاحيائية

البيولوجي الجزيئي / العملي

المرحلة الثالثة

الفصل الاول

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Lab1: preparation of laboratory solutions (concentration and Dilutions)

1. **Ratio and proportion**:

Ratio: The relationship between Two quantities using division.

Example problem:

A laboratory solution contains 58.5 grams of NaCl per liter. Express this ratio as a fraction.

Answer

The relationship can be expressed as 58.5g/1L

Proportion: is a statements that two ratios are equal.

Example problem

If there are about 100 paramecia in a 20 ml water sample, then about how many paramecia would be found in 10^3 ml of this water?

Answer

100 paramecia/ 20 ml=? /1000 ml

Cross multiply and divide: (100 paramecia) (1000 ml) = (20 ml) (?)

? = (100 paramecia) (1000 ml)/ 20 ml

? = 5000 paramecia

2. **Percent:**

Familiar type of ratio. The % sign symbolizes a fraction and the word percent means (of every hundred)

Example: 10% means 10/100 or ten out every hundred

0.1% means 0.1/100 or tenth out of every hundred

Example

300 students are surveyed to determine their favorite computer game company. Of those, 225 students prefer brand Z. This information can be expressed as a ratio, that is

225students/ 300 students

To convert this information to a percent, it is necessary to remember that percent means (out of ever hundred) the ratio 225/ 300 can be converted to a percent can be converted to percent using the logic of proportion:

225 students/ 300 students =? / 100

? =75%

3. **Density:**

The ratio between the mass (wt) and volume (vol) of a material. Thus:

Density= mass/ volume

Example

The density of glycerol at 20 C is 1.26 g/ml . what is the volume of 20.0g of glycerol?

Answer

1.26 g/ 1 ml = 20.0g/ ?

? = 20.0 g/ 1.26g

= 15.8 ml

4. **Unit conversion:**

A. Proportion method of unit conversion

If a student weighs 150 pounds, how much does he weigh in kilograms?

(1 kg = 2.2 lb)

2.2 lb/ 1 Kg = 150 lb/?

? = 68.2 kg

B. Conversion factor method of unit Conversion

A second strategy for doing conversion problems is to multiply the number to be converted times the proper conversion factor. For example: convert 2.80 kg to pounds

1 Kg = 1000 gm
1 gm = 1000 mg
1 mg = 1000 µg

1 L = 1000 ml
1 ml = 1000 µl

1 M = 1000 mM
1 mM = 1000 µM

5. Concentration and dilution

A. Concentration expression and calculation

Concentration is the amount of particular substance in a stated volume or sometimes mass of a solution or mixture.

1. (wt/ vol) fraction

$$\frac{2 \text{ gm NaCl}}{1 \text{ L water}}$$

Means that 2 gm of NaCl is dissolved in enough water so that the total volume of the solution is 1 liter.

- How could you make 300 ml of a solution that has a concentration of 10 gm of NaCl in 100 ml total volume

2. Percent (%)

- Prepare 2 % NaoH in 500 ml (% could be wt/vol or vol/vol)

- **Homework**

A solution has 5 µg/L of enzyme Q. how much enzyme Q is present in

1. 50 ml of solution 2. 100 µl of solution.

3. **Molarity**

Molarity is a concentration expression that is equal to the number of moles of a solute that are dissolved per liter of solution.

$$M = \frac{wt}{m.wt} * \frac{1000}{v}$$

- How much solute is required to prepare 1 L of 1 mM solution of $CuSo_4$ (mwt = 159.61)

4. **Part**

Parts solution tell you how many parts of each component to mix together. The parts may have any units but must be the same for all components of the mixture.

Example: A solution that is 3:2:1 of ethylene: chloroform: isoamyle alcohol is

3 parts of ethylene
2 parts of chloroform
1 parts of alcohol

- Prepare 50 ml of a solution that is 3:2:1 ethylene: chloroform: isoamyle alcohol.

B. Dilution (preparing Dilute Solutions from Concentrated Solutions)

Dilution is when one substance (often but not always water) is added to another to reduce the concentration of the first substance.

$$M1V1=M2V2$$

$$C1V1=C2V2$$

- Homework
  1. Prepare 50ml of 0.6 M NaOH from 1 M NaOh stock solution.
  2. A recipe says to mix

10 X buffer A	1µl
Solution B	2µl
Water	7µl

What is the concentration of buffer A in the final solution?

Lab2. Molarity calculation Relating to DNA and RNA.

Molarity Calculation Relating to DNA

It is necessary to know the molecular weight of a substance of interest in order to perform molarity calculations. The formula weight of a specific chemical compound is always the same so it is usually straightforward to find the formula weight of chemicals by looking at the label on their containers. **DNA is different** because its sequence and length vary depending on the source. A DNA molecule may be single stranded (SS) or double stranded (DS) and it may consist of anywhere from a few nucleotides to billions of base pairs. Therefore, there is no single MW for all DNA molecules.

1. Molecular weight of DNA molecule with known sequence and length.

	Molecular weight of Nucleotides incorporated into Nucleic Acids
A in DNA	313.22 D
C in DNA	289.18 D
T in DNA	304.21 D
G in DNA	329.22 D
A+T	313.22+ 304.21= 617.43
G+C	289.18+ 329.22= 618.4
A in RNA	329.22 D
C in RNA	305.18 D
U in RNA	306.20 D
G in RNA	345.22 D

$MW= (N_c * 289.18) + (N_a * 313.22) + (N_t * 304.21) + (N_g * 329.22) – 61.96$

2. Molecular weight of DNA molecule with unknown sequence with known length.
   A single nucleotide, on the average, has a molecular weight of 330 D and a base pair on the average has a weight of 660 D.

M.wt of DNA = (length * 330) – 61.96

(330 the average Dalton of the four nucleotide A,T,C, and G)

61.96 (removal of HPO2 and adittion of OH)

- What is the MW of a DS DNA molecules that is 100 bp long?

Lab3. Laboratory solutions for nucleic acid extraction and maintaining

Both nucleic acid extraction and Nucleic acid quantification are crucial steps to obtain reliable and quality results. The main features that are sought in DNA extraction methods include high recovery of DNA, removal of impurities and inhibitors, and high–throughput processing. Different strategies have been developed aimed to meet those demand.

DNA extraction is a routine procedure used to isolate DNA from the nucleus of cells. Scientists can buy ready-to-use DNA extraction kits. These kits help extract DNA from particular cell types or sample types. However, they can be expensive to use routinely, so many labs have their own methods for DNA extraction.

Nucleic Acid extraction steps:

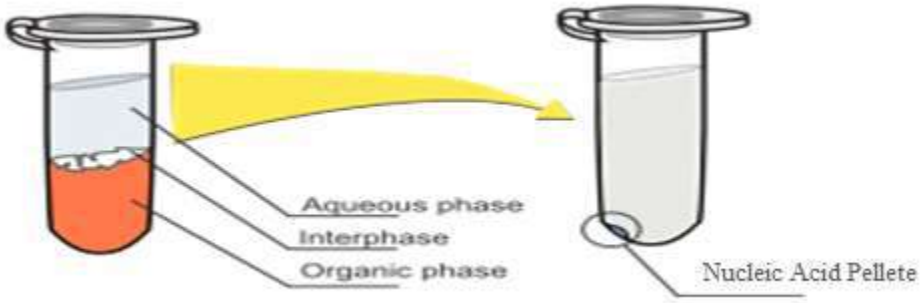
1. **Sample preparation and cell lysis.**

   The cells in a sample are separated from each other, often by a physical means such as grinding or vortexing, and put into a solution containing salts. Most lysis buffers contain buffering salts (e.g. Tris-HCl) and ionic salts (e.g. NaCl) to regulate the pH and osmolarity of the lysate. A detergent is then added, like SDS and triton X-100. The detergent breaks down the lipids in the cell membrane and nuclei. DNA is released as these membranes are disrupted.

2. **Nucleic Acid purification.**

   Organic solvents are commonly used to isolate DNA and RNA from other components of cellular extracts. A standard solvents **phenol and chloroform**, which simultaneously disrupt cell membrane and denature proteins (including endogenous nucleases). Phenol and chloroform are not water-soluble, so the mixture is centrifuged to separate it into an organic phenol/ chloroform phase and an aqueous phase. Denatured proteins move into the organic phase or the interface between the two phases. The nucleic acids remain in the aqueous phase. A low concentration of isoamyl alcohol is usually added to aid in clean separation

of the organic and aqueous phases. Phenol is relatively unstable in storage, so 8-hydroxyquinoline is usually added to phenol as anti-oxidant.



Proteinase K is an enzyme that is also added to solutions to help degrade nuclease and histones (protein associated with DNA in the chromosome).

3. **Nucleic acid precipitation**

Ethanol plays an important role in working with nucleic acids because it precipitates DNA and RNA. Physically isolating them from a solution containing other components. Nucleic acids do not lose their structural or functional integrity when isolated with phenol/ chloroform and/or phenol.

Isopropanol is sometimes substituted for ethanol when the final solution volume is needed than ethanol to precipitate DNA.

Salts is added when Nucleic acid are ethanol precipitated because relatively high concentration of monovalent cations (0.1 to 0.5 M) promote aggregation and precipitation of nucleic acid molecules. Various salts are used in different situation; however, sodium acetate is most common**.**

4. **Nucleic acid washing**

Ethanol 70% is used to remove any solutes that may be trapped in the precipitate.

5. **Nucleic Acid storage**

TE (tris- HCL and EDTA) buffer is used to store the extracted nucleic acid. Unwanted nuclease can degrade nucleic acid, particularly during long period of storage. Most nucleases require Mg^{++} as a cofactor. The level of chelators and metal ions, therefore, are

controlled in nucleic acid solutions. For example, EDTA is typically added to nucleic acid solutions during storage to reduce unwanted nuclease activity. Excess Mg ++ is added when nuclease activity is desired. The Ph of the immediate environment can have a profound effect on the structure of Nucleic acid; therefore, buffers are added to Nucleic acid solutions to protect it from denaturation. Salts like Nacl is used to maintain the ionic strength. Nucleic acids are sensitive to the ionic strength of their solutions. Denaturation of double-strand DNA is controlled in the laboratory by the ionic strength of the solution in conjugation with its temperature and PH. Consider DNA dissolved in a solution of low ionic strength. The negatively charged phosphate groups cause the two DNA strands to repel one another. In contrast, in a solution of moderate ionic strength, such as 0.4 M NaCl, Na+ ions are available that are attracted to negatively charged phosphate groups are thus neutralized. So the two DNA strands do not repel one another.

Lab 4. DNA extraction from human blood

Blood is a complex mixture of cells, proteins, metabolites, and many other substance. About 56% of human blood volume is comprised of cells, mre than 99% of which are erythrocytes. Human erythrocytes and thrombocytes (platelets, 0.5% of blood components do not contain nuclei and are therfore unsuitable for preparation of genomic DNA. The only blood cells that contain nuclei are leukocytes (0.3% of cellular blood components). Blood samples may vary widely in the number of leukocytes they contain, depending on th health of the donor. Healthy blood, for example, contains fewer than 107 leukocytes per ml, while blood from an infected donor may have a tenfold higher leukocyte concentration.

Method

Approximately 3-5 ml of blood was taken from each donor by sterile syringe and places in EDYA tubes.

1. Each blood sample was placed into 15 ml tube.
2. Te buffer was added to a volume of about 10 ml.
3. Blood and TE buffer were mixed well by inverting the tubes several times.
4. Mixture was centrifuged at 10000 rpm for 10 minute at 4C.
5. The supernatent was discarded by using a pipette to avoid losing the pellet.
6. The pellet was washed with TE buffer by repeating steps 1-4 until it is pink.
7. The supernatent was discarded by using a plastic pipette (the pellet should not be lost) and pellet was resusbended in 1 ml TBS buffer.
8. 1 ml of Lysis buffer B and 100 µl of proteinase K solution were added.
9. Tubes were incubated in waterbath 55C for 60 min with agitation
10. The samples were taken out from waterbath and 1 ml saturated phenol, and 1 ml of the mixture cloroforme: isoamyle alcohol (24:1) were added.
11. The samples were shaken 5 min by inverting the tubes.
12. The samples were centrifuged at 10000 rpm for 10 min.
13. The upper phase was transfered with plastic pipette to a new tube, the bottom phase should not be touched.
14. 2 ml of chloroforme: isoamyl alcohol (24:1) was added.

15. The samples were shaken 5 min by inverting th tubes.
16. The samples were centrifuged at 10000 rpm for 10 min.
17. The supernatent was transferred to a new tube (the bottom phase should not be touched).
18. Ammonuime acetate solution to a final concentration of 2.5 M was added and then 2.5 volumes of cold (20C) 95% ethanol.
19. The tubes wre inverted several times until DNA appeared as a white precipitate.
20. When the DNA concentration was high , DAN standars would forme a visible precipitate, which were colected into a compact mass of material that can easily be removed from the tube by spooling the DNA mass on a pasture pipette. The DAN in this manner was spoold instead of being recoverd it by centrifuge, the DNA was separated from the bluck of RNA which had been co-purifieded but remainded in solution. This eliminated the need to add exogenoous Rnase, which may be contaminated with nucleases.
21. The DNA was dried (it should not be over dried, otherwise it would be difficult to be resuspended) and it was resuspended in 2 ml of TE in 5 ml tube. And alowed to be sat until the DNA was released from the pipette tip. Once the DNA had been released, the tube was let in the room temperature for hours to allow the DNA dissolving in the buffer, and then it was stored in the deep freezer.
22. The samples which do not form a visible precipitate, the DNA was recovereded by 30 min centrifyging and resuspended in TE buffer as discriped before.

Tris-EDTA (TE) Buffer

(10 mM Tris-HCl, 1 mM Na_2EDTA, pH=8)

It was prepared by dissolving 0.2422gm of Tris-Base, 0.0744gm of EDTA in D.W, pH was adjusted to 8.0, volume completed with D.W to 100 ml, sterilized by autoclaving and stored at 4°C.

TBS buffer

(20 mM Tris-HCl, pH= 8, 150 mM NaCl)

It was prepared by dissolving 0.1211gm of Tris-Base, 0.4383gm of NaCL in D.W, pH was adjusted to 8.0, volume completed with D.W to 50 ml, sterilized by autoclaving and stored at 4°C.

B buffer

(400 mM Tris-HCl, 100 mM Na_2EDTA, pH=8, 1% SDS)

This buffer was prepared by dissolving 2.4228gm of Tris-Base, 1.86gm of Na_2EDTA, in D.W, pH was adjusted to 8.0, volume completed with D.W to 50 ml, sterilized by autoclaving then added 0.5gm of SDS.

Lab5. DNA extraction from Bacteria

Materials Required:

- Nutrient Broth
- *E. coli*
- Reagents
- TE buffer (pH 8.0)
- 10% SDS
- chloroform
- 5M Sodium Acetate (pH 5.2)
- 95% ethanol
- 70% ethanol
- Autoclaved Distilled Water
- Eppendorf tubes 2 ml
- Micropipette
- Microtips
- Microfuge

Preparation of Reagents:

1. **TE BUFFER (pH 8.0):** 10 mm Tris HCl (pH 8.0), 1 mm EDTA (pH 8.0)
2. **10% SDS:** Dissolve 10 g of SDS in 100 ml autoclaved distilled water.
3. **CHLOROFORM**
4. **5M SODIUM ACETATE:** Dissolve 41 g of sodium acetate in 100 ml distilled water and adjust pH with dilute acetic acid (pH 5.2).
5. **95% ETHANOL**
6. **70% ETHANOL**

PROCEDURE:

- 2 ml overnight culture is taken and the cells are harvested by centrifugation for 10 minutes
- 875 µl of TE buffer is added to the cell pellet and the cells are resuspended in the buffer by gentle mixing.
- 100 µl of 10% SDS is added to the cells.
- The above mixture is mixed well and incubated at 37° C for an hour in an incubator.
- 1 ml of chloroform is added to the contents, mixed well by inverting and incubated at room temperature for 5 minutes.
- The contents are centrifuged at 10,000 rpm for 10 minutes at 4° C.
- The highly viscous jelly like supernatant is collected using cut tips and is transferred to a fresh tube.
- The process is repeated once again with phenol-chloroform mixture and the supernatant is collected in a fresh tube.
- 100 µl of 5M sodium acetate is added to the contents and is mixed gently.
- 2 ml of 95% ethanol is added and mixed gently by inversion till white strands of DNA precipitates out.

- The contents are centrifuged at 5,000 rpm for 10 minutes.
- The supernatant is removed and 1ml 70% ethanol is added.
- The above contents are centrifuged at 5,000 rpm for 10 minutes.
- After air drying for 5 minutes 50 µl of TE buffer or distilled water is added.
- The concentration of DNA is determined using a spectrophotometer at 260/280 nm.
- The remaining samples are stored for further experiments.

lab6. RNA isolation

Ribonucleic acid (RNA) is a biologically important type of molecule that consists of a long chain of nucleotide units. Each nucleotide consists of a nitrogenous base, a ribose sugar, and a pohosphate. RNA is transcribed from DNA by enzymes called RNA polymerases and is generally further processed by other enzymes. RNA is central to protein synthesis. Here, a type of RNA called messenger RNA carries information from DNA to structures called ribosomes. These ribosomes are made from proteins and ribosomal RNAs, which come together to form a molecular machine that can read messenger RNAs and translate the information they carry into proteins. There are many RNAs with other roles – in particular regulating which genes are expressed, but also as the genome of most viruses.

RNA Isolation Strategies:

Efficient methodologies have been empirically derived to accommodate the expedient isolation of RNA, techniques that should be scrutinized and refined continuously. In general, these methods yield cytoplasmic RNA, nuclear RNA, or mixtures of both, commonly known as cellular RNA. Protocols for the isolation of RNA begin with cellular lysis mediated by buffers that typically fall into one of two categories: (1) those consisting of harsh chaotropic agents including one of the guanidinium salts, sodium dodecyl sulfate (SDS), urea, phenol, or chloroform, which disrupt the plasma membrane and subcellular organelles, and which simultaneously inactivate ribonuclease (RNase) and (2) those that gently solubilize the plasma membrane while maintaining nuclear integrity, such as hypotonic Nonidet P-401 (NP-40) lysis buffers.Intact nuclei, other organelles, and cellular debris are then removed from the lysate by differential centrifugation. The reliability of this approach is often dependent on the inclusion of nuclease inhibitors in the lysis buffer and careful attention to the handling andstorage of RNA so purified.

Diethylpyrocarbonate (DEPC).

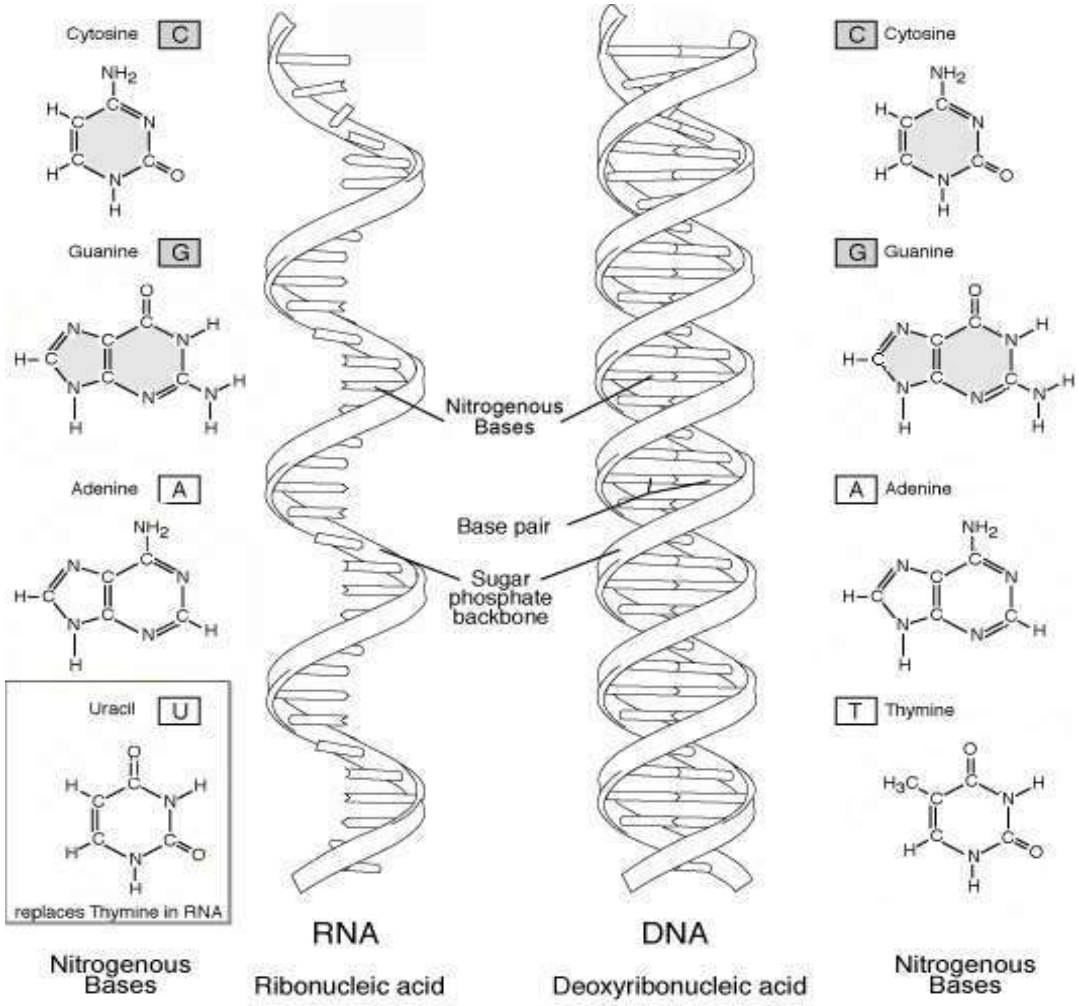
Is used in the laboratory to inactivate RNase enzymes in water and on laboratory utensils. It does so by the covalent modification of histidine, lysine, cysteine, and

tyrosine residues. Water is usually treated with 0.1% v/v DEPC for at least 2 hours at 37 °C and then autoclaved (at least 15 min) to inactivate traces of DEPC. Inactivation of DEPC in this manner yields CO2 and ethanol. Higher concentrations of DEPC are capable of deactivating larger amounts of RNase, but remaining traces or byproducts will modify purine residues in RNA and may inhibit further biochemical reactions such as in vitro translation.

Comparison between DNA and RNA.

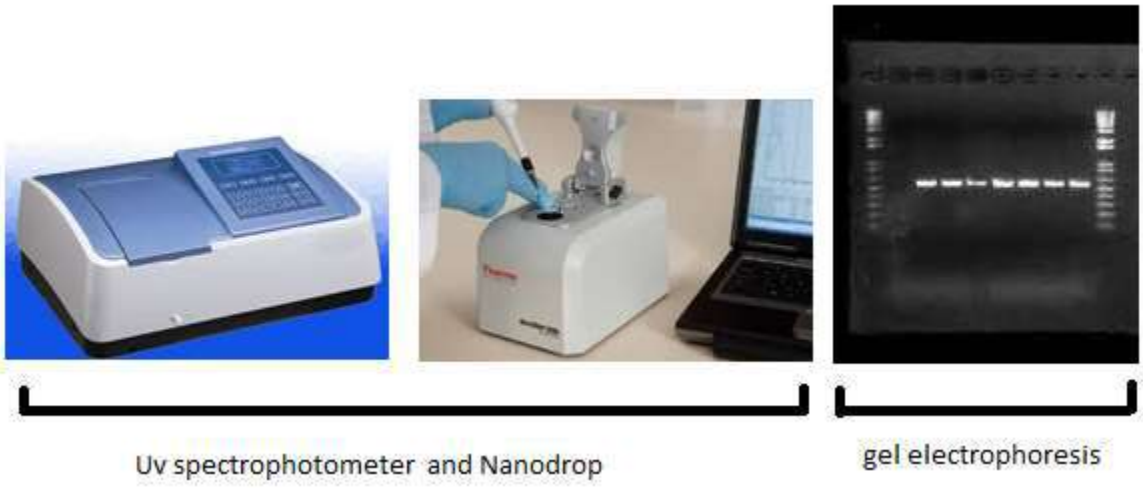
	DNA	**RNA**
Stability:	Deoxyribose sugar in DNA is less reactive because of C-H bonds. Stable in alkaline conditions. DNA has smaller grooves where the damaging enzyme can attach which makes it harder for the enzyme to attack DNA	Ribose sugar is more reactive because of C-OH (hydroxyl) bonds. Not stable in alkaline conditions. RNA on the other hand has larger grooves which makes it easier to be attacked by enzymes
Pairing of Bases:	A-T(Adenine-Thymine), G-C(Guanine-Cytosine)	A-U(Adenine-Uracil), G-C(Guanine-Cytosine)
Difference:	1.Found in nucleus 2.the genetic material 3. sugar is dexyribose 4. Bases are A,T,C,G	1.Found in nucleus and cytoplasm
Predominant Structure:	Typically a double-stranded molecule with a long chain of nucleotides	A single-stranded molecule in most of its biological roles and has a shorter chain of nucleotides
Types:	Single	1) mRNA (carries DNA message to cytoplasm) 2)tRNA (carries

	DNA	RNA
		amino acids to mRNA and Ribosomes) 3)rRNA(Ribosomal RNA, workbench for protein synthesis)
Unique Features:	The helix geometry of DNA is of B-Form. DNA is completely protected by the body i.e. the body destroys enzymes that cleave DNA. DNA can be damaged by exposure to Ultra-violet rays	The helix geometry of RNA is of A-Form. RNA strands are continually made, broken down and reused. RNA is more resistant to damage by Ultra-violet rays
Stands for:	Deoxyribonucleic acid	Ribonucleic acid
Definition:	A nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms	A nucleic acid polymer that plays an important role in the process that translates genetic information from deoxyribonucleic acid(DNA) into protein products
Job/Role:	Medium of long-term storage and transmission of genetic information	Acts as a messenger between DNA and the protein synthesis complexes known as ribosomes
Bases & Sugars:	DNA is a long polymer with a deoxyribose and phosphate backbone and four different bases: adenine, guanine, cytosine and thymine	RNA is a polymer with a ribose and phosphate backbone and four different bases: adenine, guanine, cytosine, and uracil


Cytosine C
Guanine G
Adenine A
Uracil U
replaces Thymine in RNA
Nitrogenous Bases
Nitrogenous Bases
Base pair
Sugar phosphate backbone
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid
C Cytosine
G Guanine
A Adenine
T Thymine
Nitrogenous Bases

Lab7. Determining DNA concentration and purity.

The most comprehensive way to evaluate DNA concentration and purity is to use both UV spectrophotometric measurements and agarose gel electrophoresis.



UV spectrophotometric measurement of DNA concentration and purity.

DNA itself, and most of the common contaminants found in DNA preps, have absorbance in the region 230nm to 320nm so measurement of the absorbance in this region allows measurement of the DNA concentration and provides information about the contaminant levels. The most important wavelengths to note are:

- **230nm**: Guanidium salts (used to facilitate DNA binding to silica columns) and phenol (used in phenol/chloroform extractions) absorb strongly at 230nm, therefore high absorbance at this wavelength can be indicative of carry-over of either of these compounds into the sample.
- **260nm:** DNA absorbs light most strongly at 260nm so the absorbance value at this wavelength (called A_{260}) can be used to estimate the DNA concentration. Nucleic acids absorb in the ultraviolet region of the spectrum due to the conjugated double bond and ring systems of the constituent purines and pyrimidines.
- **280nm:** Since tyrosine and tryptophan residues absorb strongly at this wavelength, the absorbance at 280nm is used as an indicator of protein contamination.
- **320nm:** A_{320} provides a general measurement of the turbidity of the sample and is normally subtracted from the A_{260} value as a background reading for the calculation of DNA concentration, but excessive values may indicate non-specific contamination.

DNA concentration can be determined by:

1. 1 OD_{260} unit = 50 µg/ml or

   50 ng/µl

2. Unknown diluted µg/ml = 50 µg/ml x Measured A260 x dilution factor.

Nucleic acid	Concentration µg/ml per A 260
DS DNA	50
SS DNA	33
SS RNA	40

In most DNA preparation, the final step is the separation of DNA from proteins. Carryover proteins during DNA prep could led to problems with subsequent operations, such as cutting with restriction endonuclease. Assessment of DNA purity is therefore important.

The most commonly used assay for DNA purity is:

$$\text{DNA purity} = A_{260} / A_{280}$$
$$= 1.8$$

The most commonly used assay for RNA purity is:

$$\text{RNA purity} = A_{260} / A_{280}$$
$$= 2$$

Lab8. Gel Electrophoresis of DNA

This technique separates and purifies fragments of DNA or RNA as well as proteins. The basic idea of **electrophoresis** is to separate the molecules based on their electrical charge. Electrically positive charges attract negative charges and repel other positive charges. Conversely, negative charges attract positive charges and repel other negative charges. Two electrodes, one positive and the other negative, are connected up to a high voltage source. Positively charged molecules move towards the negative electrode and negatively charged molecules move towards the positive electrode. Since DNA carries a negative charge on each of the many phosphate groups making up its backbone, it will move towards the positive electrode during electrophoresis.

Most DNA is separated using **agarose gel electrophoresis**. **Agarose** is a polysaccharide extracted from seaweed. When agarose and water are mixed and boiled, the agarose melts into a homogeneous solution.

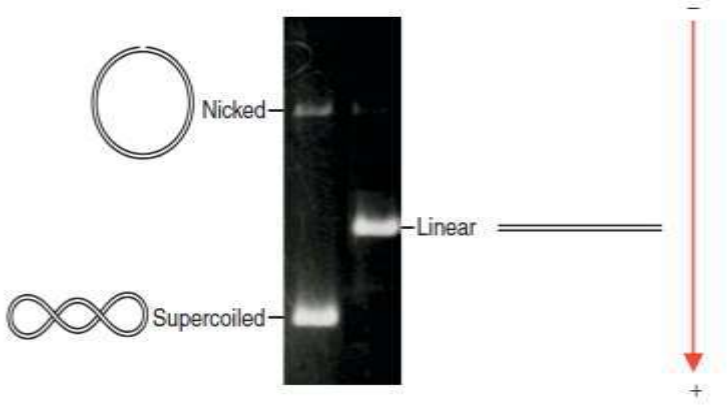
As the solution cools, it gels to form a meshwork, which has small pores or openings filled with water. The cooled gel looks much like a very concentrated mixture of gelatin without the food coloring. The pore size of agarose is suitable for separating nucleic acid polymers consisting of several hundred nucleotides or longer. Shorter fragments of DNA as well as proteins are usually separated on gels made of **polyacrylamide Gel (PAGE)**. The meshwork formed by this polymer has smaller pores than agarose polymers. PAGE is a powerful technique in the analysis of DNA molecules, and is able to very effectively separate DNA molecules that differ in size by as little as a single base pair. This high level of resolution makes PAGE ideal for the analysis of DNA sequence. The technique is, however, limited to relatively small DNA molecules (less than 1000 bp in length). Large DNA molecules are unable to enter the pores of the polyacrylamide and are consequently not separated by the gel.

As the DNA molecules move through the gel they are hindered by the meshwork of fibers that make up the gel. The larger molecules find it more difficult to squeeze through the gaps but the smaller ones are slowed down much less. The result is that the DNA fragments separate in order of size , the rings of plasmid DNA will move farther in the gel than the chromosome.

THE RATE OF MIGRATION OF DNA THROUGH AGAROSE GELS

The following factors determine the rate of migration of DNA through agarose gels:

1. **The molecular size of the DNA**: Larger DNA molecules migrate more slowly than small molecules because of greater frictional drag.
2. **The concentration of agarose**: linear DNA fragments of a given size migrates at different rates through gels containing different concentration of agarose.
3. **The conformation of the DNA (topology**): Super helical circular, nicked circular, and linear DNA migrate through agarose gels at different rates. Plasmid DNA isolated from *E. coli* cells is invariably negatively supercoiled closed-circular molecules. These are relatively compact structures that run quickly through agarose gels. If one strand of the plasmid double helix becomes broken (nicked) then the supercoiling within the plasmid will be lost, and the more open structure of the relaxed plasmid will migrate more slowly through an agarose gel. If the same plasmid is treated with a restriction enzyme that cleaves it once, then this linearized DNA will run with a mobility intermediate between those of the supercoiled and the nicked molecules. Therefore, DNA molecules that all contain precisely the same number of base pairs can run in several different locations on an agarose gel depending upon the topology of the DNA.



4. **The presence of ethidium bromide in the gel and electrophoresis buffer**: Intercalation of ethidium bromide causes a decrease in the negative charge of the double stranded and increase both its stiffness and length.

5. **The applied voltage**: The effective range of separation in agarose gels decreases as the voltages increased.

6. **The type of agarose**: The electrophoresis mobility of DNA is affected by the composition and ionic strength of the electrophoresis buffer, in the absence of ions electrical conductivity is minimal and DNA migrates slowly. In buffer of high ionic strength electrical conductance is very efficient and significant amounts of heat are generated and the gel melts and the DNA denatures.

The required equipment for conducting agarose gel electrophoresis:

1. Electrophoresis chamber.
2. Power supply.
3. Gel casting trays.
4. Combs.
5. Trans illuminator (an ultraviolet lightbox).

Required buffers and dye

1. **Electrophoresis buffer (10x)**, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
2. **Loading buffer (6x)**, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
3. **Ethidium bromide (10 mg/ml in final con. 0.5 µg/ml)**, a fluorescent dye used for staining nucleic acids is able to intercalate between the stacked base pairs of DNA, Ethidium bromide will bind very efficiently to double-stranded DNA, but less so to single-stranded DNA and RNA because of the relative lack of base stacking.
4. Agarose gel 0.7-1% in 1X TBE**.**

Casting the Agarose Gel

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
2. Place a well-former template (comb) in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.
3. Use a 250mL flask to prepare the gel solution. Add the following components to the flask.
    a. 0.24 g of Agarose
    b. 0.6 mL of concentrated buffer solution
    c. 29.4 mL of distilled water.
4. Swirl the mixture to dispense clumps of agarose powder
5. With a marking pen, indicate the level of the solution volume on the outside of the flask.
6. Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any dissolved particles
7. Cool the agarose solution to 55oC with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask previously.
8. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.
9. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

Preparing the Chamber

1. After the gel is completely solidified, carefully and slowly remove the rubber dams.
2. Remove the comb slowly by pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.
3. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
4. Fill the electrophoresis apparatus chamber with the required volume of diluted buffer for the specific unit you are using.
5. Make sure the gel is completely covered with buffer.
6. Proceed to loading the samples and conducting electrophoresis.

Loading Samples:

1. The amount of sample that should be loaded is 35-38 microliters.

Running Samples:

1. After the samples are loaded, carefully snap the cover down onto the electrode terminals. Make sure that the negative and positive color-coded indicators on the cover and apparatus chamber are properly oriented.
2. Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).
3. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor.
4. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.
5. After approximately 10 minutes, you will begin to see separation of the colored dyes.
6. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.
7. Document the gel results.

<u>If you see faint or no bands on the gel:</u>

- There was insufficient quantity or concentration of DNA loaded on the gel.
- The DNA was degraded.
- The DNA was electrophoresed off the gel.
- Improper W light source was used for visualization of ethidium bromide-stained DNA.

<u>If you see smeared DNA bands:</u>

- The DNA was degraded. Avoid nuclease contamination.
- Too much DNA was loaded on the gel. Decrease the amount of DNA.

- Improper electrophoresis conditions were used.
- The DNA was contaminated with protein.

If you see anomalies DNA band migration:

- Improper electrophoresis conditions were used. Do not allow voltage to exceed ~20 V/cm. Maintain a temperature <30° C during electrophoresis. Check that the electrophoresis buffer used had sufficient buffer capacity.
- The DNA was denatured